

Physiology / Physiologie

Thiocyanate effects on thyroid function of weaned mice

Hela Ghorbel^a, Hamadi Fetoui^{a,1}, Amira Mahjoubi^{a,1},
Fadhel Guerhazi^b, Najiba Zeghal^{a,*}

^a *Animal Physiology Laboratory, Faculty of Sciences, Soukra road – Km 3.5, BP 802, 3018 Sfax, Tunisia*

^b *Nuclear Medicine Service, CHU Habib-Bourguiba, 3029 Sfax, Tunisia*

Received 23 October 2007; accepted after revision 30 January 2008

Available online 4 March 2008

Presented by Pierre Buser

Abstract

The aim of this study was to determine the effects of thiocyanate on thyroid function in weaned mice. At this developmental period, induction and reversibility of thiocyanate effects have not yet been studied. In the present work, adult female mice were given thiocyanate [SCN^- (1 gL^{-1})] in their drinking water from the 15th day of pregnancy until either the 25th (group B) or the 15th day (group C) after parturition. During five days after weaning, water and food consumptions of treated mice (group B) were $42.2 \pm 1.2\%$ and $56.4 \pm 0.9\%$, respectively, less than those of the controls (group A). On the sacrifice day (the 25th day after birth), body weight, thyroid iodine content and thyroid hormone levels (FT_4 and FT_3) decreased by $10.4 \pm 3.0\%$, $40.6 \pm 2.3\%$; $18.7 \pm 2.3\%$ and $18.1 \pm 1.3\%$, respectively. Plasma TSH increased by $30.6 \pm 1.7\%$ along with the hypertrophy of thyroid glands ($52.6 \pm 3.1\%$). We have observed a hypertrophy of follicle cells and a decrease in colloid volume within histological slides. After SCN^- withdrawal (group C), partial or total recovery were noted in all parameters studied. We concluded that hypothyroidism effects added to the weaning event affected greatly thyroid function and behaviour of mice; these would be largely reversed by withdrawing thiocyanate treatment for a period of ten days. **To cite this article:** H. Ghorbel et al., C. R. Biologies 331 (2008).

© 2008 Published by Elsevier Masson SAS on behalf of Académie des sciences.

Résumé

Effets du thiocyanate sur la fonction thyroïdienne des jeunes souris sevrées. L'objectif du présent travail consiste à étudier l'induction et la réversibilité d'action du thiocyanate sur la fonction thyroïdienne chez la jeune souris en période du sevrage. Des souris gestantes adultes ont été réparties en trois groupes. Le groupe A représente des témoins, tandis que les groupes B et C sont constitués par des souris traitées à partir du 15^e jour de gestation au thiocyanate [SCN^- (1 gL^{-1})], administré par la boisson des mères pendant 31 et 21 jours, respectivement. Durant les cinq jours qui suivent le sevrage, nous avons noté une diminution des prises quotidiennes en aliment solide ($42,2 \pm 1,2\%$) et en boisson ($56,4 \pm 0,9\%$) comparativement à celles des témoins. Toutes les jeunes souris ont été sacrifiées à l'âge de 25 jours. Nous avons obtenu chez les jeunes souris traitées du groupe B une diminution du poids corporel ($10,4 \pm 3,0\%$), du contenu en iode ($40,6 \pm 2,3\%$) et des taux plasmatiques en FT_4 et FT_3 ($18,7 \pm 2,3\%$ et $18,1 \pm 1,3\%$, respectivement). Chez les souris de ce groupe, le contenu plasmatique en TSH a augmenté de $30,6 \pm 1,7\%$, ce qui va de pair avec une hypertrophie de la thyroïde ($52,6 \pm 3,1\%$). Ces perturbations biochimiques sont associées à des modifications de l'aspect histologique de la thyroïde. L'arrêt du traitement, dix jours avant le sacrifice des souris du groupe C, a induit une réversibilité

* Corresponding author.

E-mail address: najiba.zeghal@tunet.tn (N. Zeghal).

¹ These authors contributed equally to this work.

partielle ou totale des paramètres biochimiques et histologiques de la fonction thyroïdienne. Nous pouvons conclure que les effets de l'hypothyroïdisme, associés à ceux du sevrage, modifient fortement le fonctionnement thyroïdien et le comportement des souris, lesquels peuvent être corrigés par un arrêt de traitement pendant une période de dix jours. *Pour citer cet article : H. Ghorbel et al., C. R. Biologies 331 (2008).*

© 2008 Published by Elsevier Masson SAS on behalf of Académie des sciences.

Keywords: Thiocyanate; Thyroid hormones; Weaned mice; TSH; Histology

Mots-clés: Thiocyanate; Hormones thyroïdiennes; Souris sevrées; TSH; Histologie

1. Introduction

Thyroid dysfunction could be induced by various factors such as ionizing radiations [1], pollution [2–4] malnutrition [5], antithyroid drugs [6,7] dietary goitrogens [8], and iodine-deficiency [9]. Children born in iodine-deficient areas are at risk of neurological disorders and mental retardation because of the combined effects of maternal, foetal, and neonatal hypothyroxinemia [10]. In some parts of African countries like Congo, Uganda, and Kenya, the prevalence of goitre exceeds 81% [11]. Although iodine deficiency is the main factor in the aetiology of endemic goitre, the additional role of goitrogens has been shown or suspected in areas such as Congo and Sudan, where goitre is endemic [12]. In these regions, people are exposed to thiocyanate overload provided by consumption of cassava roots, which are used as a staple food and contain the cyanogenic glucoside linamarine [13]. Linamarine metabolism produces thiocyanate (SCN^-), a well-known goitrogen [14]. SCN^- ion competes with iodide (I^-) trapping, iodide oxidation, and binding to thyroglobulin at the level of the thyroperoxidase enzyme [15]. Indeed, the SCN^- and the iodide share some common physiochemical properties, and both are oxidized by the peroxidase enzyme [16].

In Africa, poverty and food scarcity due to drought and war force consumption of incomplete detoxified cassava [17]. The SCN^- overload originating from consumption of poorly detoxified cassava is considered a contributing factor responsible for the increase of the goitre rate [18].

Life-style-related factors such as smoking are considered another common source of thiocyanate contamination. In fact, cigarette smoke contains several toxins such as hydrogen cyanide and 2,3-hydroxypyridine [19, 20]. In the whole world, tobacco arises as a very serious problem. According to the World Health Organization (WHO) report (2002) dealing with worldwide smoking statistics, about one third of the male adults

in global population smoke; smoking is on the rise in the developing world, but is falling in developed nations. Among people of the developing world, tobacco consumption is rising by 3.4% per year [21]. Cigarette smoke containing HCN is rapidly absorbed by the lungs, the systemic circulation spreading out in the entire organism by passing the portal circulation [22]. HCN is detoxified by a conversion through an intramitochondrial enzyme, the rhodenase, to the less toxic compound, the thiocyanate [23]. Consequently, smoking increases greatly SCN^- levels in the serum [16].

Human thiocyanate contamination can be also the result of pesticide use. For example, aminotriazole, a systemic non-selective herbicide, is often associated with ammonium thiocyanate, which enhances its activity [24].

Manufacturing activities releasing hydrogen cyanide include electroplating, metal mining, metallurgy, and metal cleaning process; these are considered the most important cause of thiocyanate contamination in workers [25,26].

Thiocyanate can be transmitted from dams to pups during the lactating period via breast milk feeding. Indeed, SCN^- effects were assessed on different experimental species, such as rats [7], mice [6], and infants [22], during the lactating period. However, there are no previous investigations dealing with thiocyanate consumption and thyroid function of weaned young mice during the developmental period.

The objective of our work was to study the effects of weaning period on the thyroid function of thiocyanate-treated young mice, issued from thiocyanate-treated mothers. Weaning is a very critical period for pups' behaviour and maturity (water and food consumption), especially if we take in consideration the fact that hypothyroidism provokes a delay in nervous system development and behaviour maturity, enhancing more difficulties for adaptation to the new life conditions, in the lack of mother care.

2.3. Determination of morphological and biochemical parameters

After anaesthesia with chloral hydrate by intraabdominal way, body weights of young mice were measured. Femurs (right or left) were carefully dissected out for length and weight determination ($n = 48$). Blood samples were collected from the brachial artery of pups and plasma samples were withdrawn after centrifugation at 2200 g for 15 min and kept at -20°C . Only eight samples of plasma were randomly chosen for the determination of free T_4 , free T_3 and TSH by radioimmunoassay, using commercial kits from Immunotech, France (refs: 1363 (FT₄); 1579 (FT₃)), and for that of TSH using a rat-specific TSH kit supplied by IBL-Germany (ref.: AHR001).

2.4. Chemical and histological studies

Thyroid glands ($n = 42$) were removed from pups, they were weighed and kept at -20°C until biochemical analysis. Eight thyroid glands were randomly selected for iodine content determination after alkaline mineralization, according to Sandell and Kolthoff's method [27].

Six thyroid samples were randomly selected for light microscopy. They were taken with a piece of trachea and fixed in a Bouin solution, embedded in paraffin and serially sectioned at 5 μm . Then, the sections were stained with hematoxyline eosin (HE) [29].

2.5. Statistical analysis

Comparisons of mean values between mice treated groups (B and C) and control group (A) or between treated mice (group C and group B) were made using ANOVA followed by Student's t -test. Statistical significance was defined as a P value of less than 0.05. Values were expressed as the means followed by standard deviation.

3. Results

3.1. Growth and feeding

3.1.1. Body weight variation

The dose of SCN^- (Table 2), administered to group B, provoked growth retardation of mice comparatively to group A. Indeed, the body weight was decreased by $10.4 \pm 3.0\%$ (Fig. 1). When thiocyanate was eliminated from the mothers' drinking water on the 15th day following parturition (mice of group C), a partial recovery

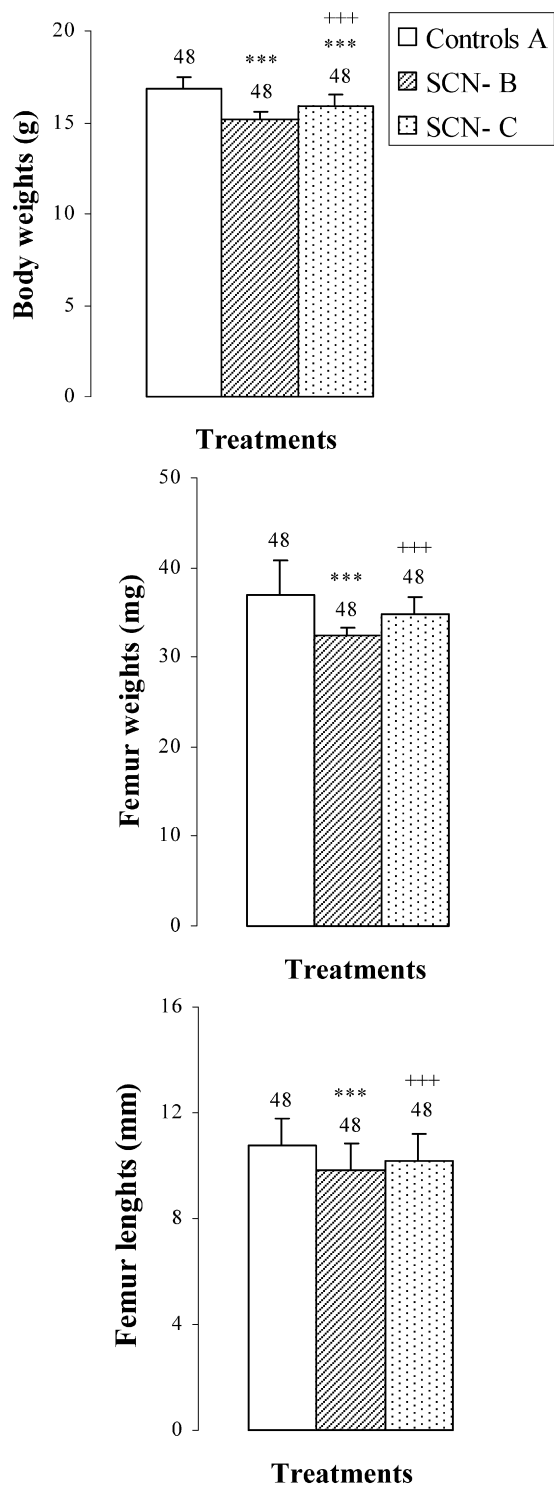


Fig. 1. Body weights, femur weights and lengths of 25-day-old mice, controls (group A) and treated with thiocyanate [SCN^- (1 g/L)] given to mothers' drink from day 15 of pregnancy until either the 25th day (group B) or the 15th day (group C) after delivery. Treated B or C vs. controls A: *** $p \leq 0.001$. Treated C vs. treated B: +++ $p \leq 0.001$. The number of determinations is represented above the columns.

was obtained in body weight after ten days ($6.0 \pm 0.9\%$) comparatively to animals of group B (Fig. 1). This recovery occurred without reaching control values.

3.1.2. Femur length and weight variations

The femur weights and lengths of mice of group B were decreased by $20.1 \pm 2.3\%$ and $8.8 \pm 1.2\%$, respectively, comparatively to those of controls (Fig. 1). In group C, bone growth was partially recovered in comparison with group B ($+10.8 \pm 1.6\%$ in femur lengths and $+24.9 \pm 2.5\%$ in femur weights). This improvement occurred without reaching control values (group A) (Fig. 1).

3.1.3. Food and water consumption

The decrease in body weight was associated with a reduction in food ($-56.4 \pm 0.9\%$) and water ($-42.2 \pm 1.2\%$) consumptions and in ingested iodine quantities ($-53.0 \pm 0.9\%$) (Table 2). Compared to group B, group C showed an improvement in water and food consumptions by ($+36.4 \pm 1.8$) and ($+51.8 \pm 2.3\%$), respectively (Table 2).

3.2. Thyroid gland weight variations

An hypertrophy of the thyroid gland by $+52.6 \pm 3.1\%$ was noted in pups treated with SCN^- (group B) (Fig. 2), probably resulting from the increase ($+31.0 \pm 1.7\%$) of TSH: plasma thyroid stimulating hormone (Fig. 2). After SCN^- withdrawal (group C), thyroid gland weight decreased by ($-35.3 \pm 2.6\%$) comparatively to animals of group B (Fig. 2). Plasma TSH levels decreased by $87.3 \pm 4.3\%$ ($P \leq 0.001$) and by $81.8 \pm 6.2\%$ ($P \leq 0.001$) when compared to groups B and A, respectively (Fig. 2).

3.3. Thyroid hormone levels

The thyroid hormone levels FT_4 and FT_3 in plasma were decreased by $18.7 \pm 2.3\%$ and $18.1 \pm 1.3\%$, respectively, in mice of group B comparatively to those of group A (Fig. 2). The reduction of plasma thyroid hormone levels was the result of the decrease in daily-ingested iodine ($-53.1 \pm 0.6\%$) and thyroid iodine content by ($-40.6 \pm 2.3\%$) (Table 2 and Fig. 2). After SCN^- withdrawal, group C showed an increase in thyroid iodine contents ($+29.1 \pm 1.8\%$) and in plasma thyroid hormone levels of FT_4 by $15.8 \pm 1.2\%$ and of FT_3 by $13.4 \pm 0.8\%$ when compared to those of group B (Fig. 2). Daily-ingested iodine was also improved by ($+49.0 \pm 2.3\%$) in group C, comparatively to group B (Table 2), while compared to animals of group A, mice

of group C presented a total restoration of plasma FT_4 levels, although thyroid iodine contents and plasma FT_3 levels of group C were lower than those of group A and were respectively increased by $+16.0 \pm 0.9\%$ ($P \leq 0.01$) and $+5.4 \pm 0.8\%$ ($P \leq 0.001$) (Fig. 2).

3.4. Histological modifications

The morphological and biochemical perturbations were confirmed by the histological study of thyroid glands. Indeed, thyroids of group B presented closed follicles, follicular cell hyperplasia, an increase in follicular number and vascularity as well as a decrease in colloid volume (Fig. 3, photo B). However, in controls (group A), the majority of follicles were larger than those in treated mice (B and C), lined by more flattened epithelium and filled with a deeper staining colloid (Fig. 3, photo A). Ten days after SCN^- withdrawal (group C), the histological aspect of the thyroid glands was improved comparatively to animals of group B, with an increase in colloid volume and a decrease of follicular cell hypertrophy (Fig. 3, photo C).

4. Discussion

Weaning is one of the most important events in the early stage of life. After weaning, pups become nutritionally and behaviourally independent of their dam, so the weaning procedure influences their physiological and neurobehavioural development [30].

In this study, we noted important thyroid hormone changes within the pup, which occurred during the weaning time period. It is well known that two hormones, specifically thyroxin (T_4) and corticosterone, show developmental surges prior to weaning initiation [31,32]. These hormones have been shown to induce maturational changes in various organ systems during the third postnatal week [33]. Moreover, it was previously demonstrated that hypothyroidism affects the timing of weaning [34], whereas weaning effects on hypothyroid offspring physiology and behaviour are yet less well understood.

In the present study, thiocyanate treatment of group B mice provoked a decrease in body weight, femur growth, thyroid iodine content, and plasma thyroid hormone levels. Because the iodine is the precursor of thyroid hormones' biosynthesis, the transport and the concentration of iodine within the thyroid gland represent the first step in the production of the iodine content of thyroid hormones [35]. Indeed, iodide uptake occurs across the basolateral membrane of polarized thyroid follicular cells

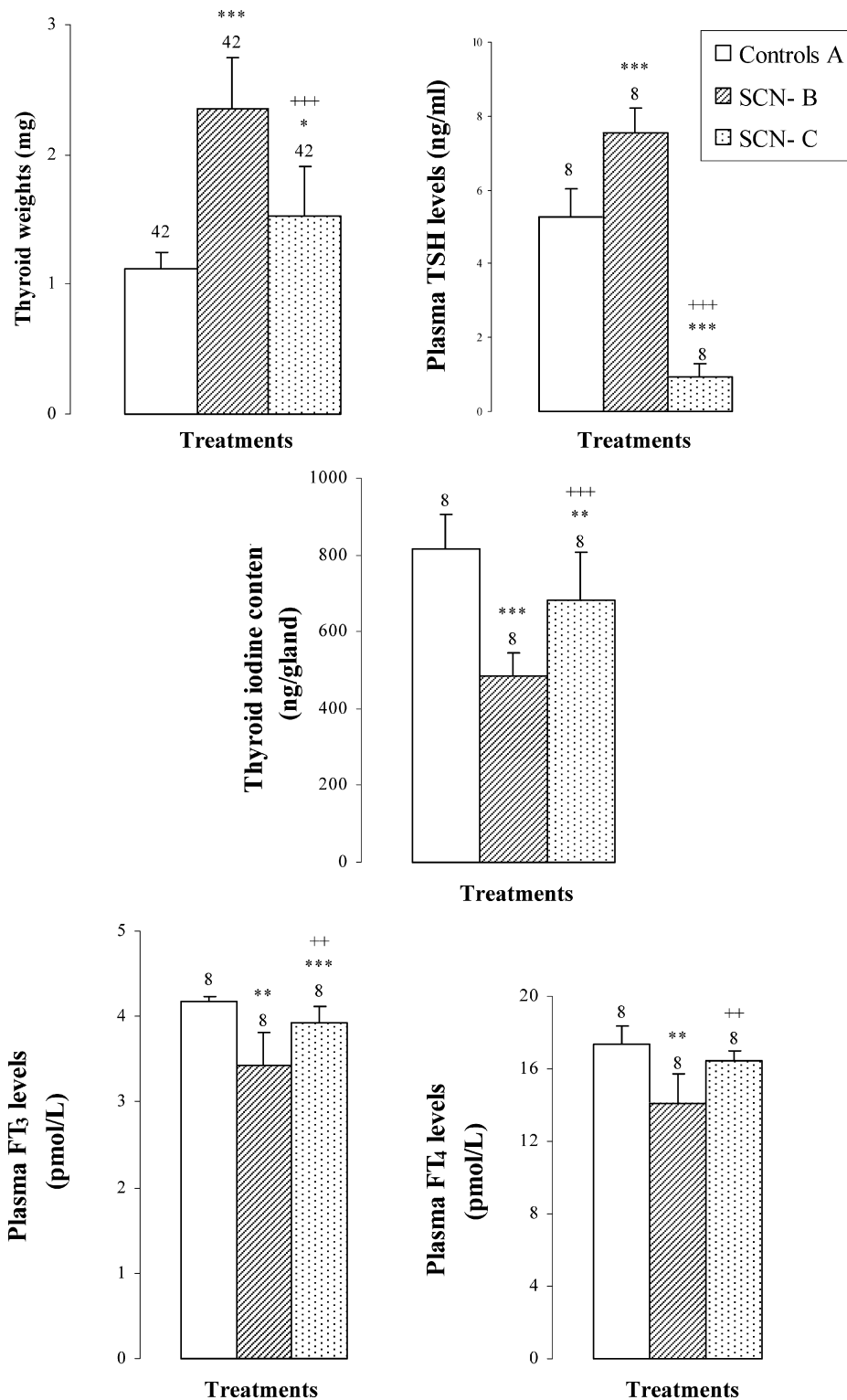


Fig. 2. Thyroid weights, plasma TSH levels, thyroid iodine content, and plasma thyroid hormone (FT₄, FT₃) levels of 25-day-old mice, controls (group A) and treated with thiocyanate [SCN⁻ (1 g/L)] given to mothers' drink from day 15 of pregnancy until either the 25th day (group B) or the 15th day (group C) after delivery. Treated B or C vs. controls A: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Treated C vs. treated B: ++ $p \leq 0.01$; +++ $p \leq 0.001$. The number of determinations is represented above the columns.

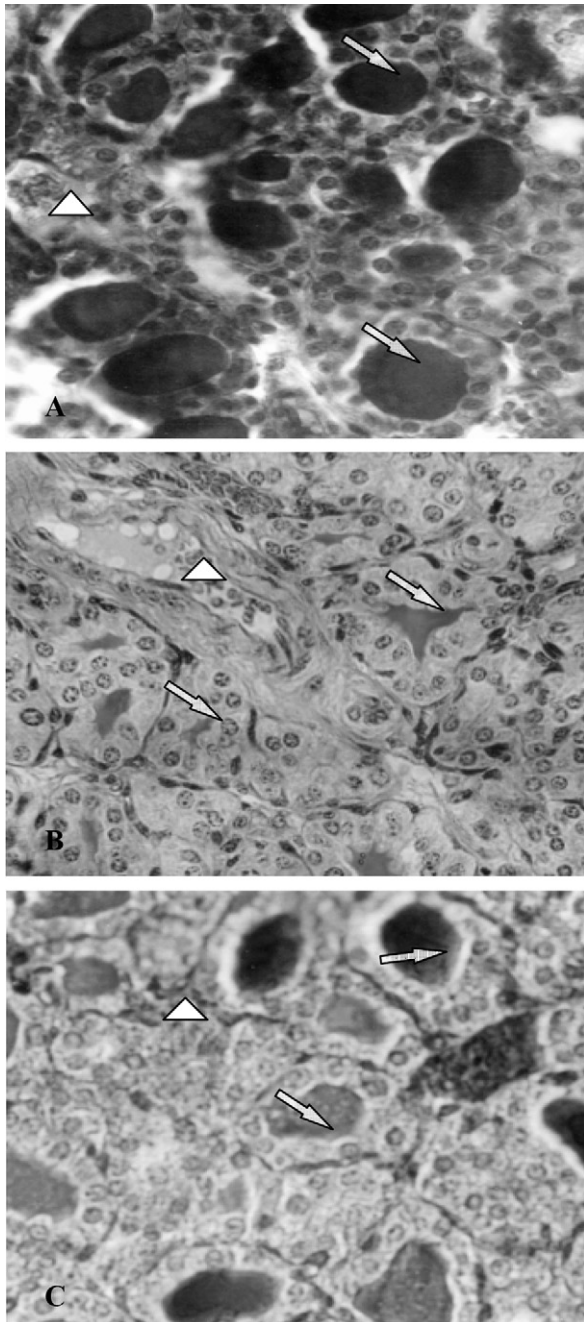


Fig. 3. Histological thyroid sections of 25-day-old mice, controls (group A) and treated with thiocyanate [SCN^- (1 g/L)] given to mothers' drink from day 15 of pregnancy until either the 25th day (group B) or the 15th day (group C) after delivery. \Rightarrow : Arrows indicate thyroid follicles; \blacktriangleright : arrows indicate blood vessels.

and is an active transport driven by inwardly directed Na^+ gradient [36]. In rats, the activity of sodium iodide symporter (NIS), an intrinsic membrane-associated protein cloned and characterized, was shown to be under

the control of TSH [37]. Thiocyanate and iodide (I^-) have a similar charge and a comparable ionic volume, and the SCN^- could be transported by the NIS system [38]. Furthermore, similarly to iodide trapping, the SCN^- catch by the thyroid cell is increased in the presence of the TSH [39] and the intrathyroidal metabolism of SCN^- also increased after TSH stimulation. Hence, the SCN^- would be rapidly oxidized by the thyroperoxidase (TPO) enzyme in the presence of H_2O_2 . Therefore, as it was recently demonstrated by Contempré et al. [16], the SCN^- is not accumulated in the thyrocyte, and it ultimately generates an I-deficient thyroid with a decrease in thyroid hormones' synthesis that would induce growth retardation [16]. Actually, many other studies have shown the stimulation of bone growth by thyroid hormones that have a direct effect on longitudinal bone growth in both humans and rats [40,41] in addition to their direct effect on the nervous system's maturation [42].

During the five days of mice weaning, we have noted an important daily decrease in food and water consumptions and in iodine intake. These behavioural perturbations could be the result of the stress caused by weaning when the totally mother-dependent pups become self-autonomous mice. Most of the physiological functions were modified in correlation with an important maturation of organs (increase in intestine size, acceleration of bone ossification, autonomous thermoregulation...). Moreover, young mice have to find their food and respond to external stimuli [43]. Furthermore, Goody and Kitchen [44] have demonstrated that weaning rat pups at day 21 activate the δ -opioid receptor that mediates swim-stress-induced analgesia (swim SIA), while Muhammad and Kitchen [45] have shown that a delay of weaning until the 25th day beyond the normal day of weaning was able to halt the μ - to δ -opioid receptor transition and the mediation of swim SIA by 5 to 10 days. The recent study of Hickey et al. [46] showed that the abrupt weaning of calves is a stressor that also increases the physiological measures of stress hormones. These data correlates with our findings, showing that while adding SCN^- in the drinking water, a feeding decrease and behavioural perturbations occurred.

On the sacrifice day of mice treated during the full period (group B), an increase in thyroid gland weights was obtained. This result can be explained by a feedback to the hypothalamo-hypophysis axis, indicating the low levels of FT_4 and FT_3 in the blood; hence, more thyroid-stimulating hormone (TSH) was released in order to increase the production of these hormones. Consequently, hypothyroidism and hypertrophy of the thyroid gland were evidenced. These states of the thy-

roid gland, observed in group B, were the result of the continuous SCN^- exposure from the neonatal period until the weaning period. Indeed, SCN^- is transmitted from dams to foetus during pregnancy by placenta [47, 48] and from mothers to pups during the lactating period by breast milk [6,22,49,50]. After weaning, young mice consumed directly SCN^- in the drinking water until the sacrifice day. Recent studies showed that hypothyroidism is associated with modest pituitary enlargement in humans, and several case reports of pituitary enlargement sufficient to present visual impairment through comparison of the optic chiasma have been published [51,52]. These data correlate with an important TSH secretion under hypothyroid state, as it was found in our study.

Histological changes observed in thyroid slides of group B were characterized by closed follicles, follicular cell hyperplasia, an increase in follicular number, and vascularity, as well as a decrease in colloid volume. These data confirmed previous results by Soussia and collaborators [7], who had demonstrated that in the case of rat species, thiocyanate treatment provoked follicular cell hyperplasia and a decrease in colloid volume. Moreover, these authors observed, on the periphery of follicular cavities, the presence of empty vacuoles resulting from colloid resorption. This event was not observed on mice histological slides.

When SCN^- was removed from the drink of group C mothers at the 15th day after delivery, the young s' body and femur growth rates, the thyroid iodine content and plasma thyroid hormone levels increased in comparison with those of group B, but did not reach control values (group A).

Improvement of all biochemical parameters might be explained, first, by an amelioration of the mothers' milk iodide content before the weaning day (21st day). Indeed, thiocyanate treatment during the lactating period inhibited the two effects of prolactine on iodide and NIS accumulation by the mammary gland [53]. During this period, thiocyanate inhibited iodide transporter activity as well as bearing functions like a general metabolic inhibitor [53]. Furthermore, results of Ben Hamida et al. [6] showed that after 12 days of thiocyanate withdrawal from drinking water during the lactating period, body weight and plasma thyroid hormone levels of both mothers and pups increased. Second, after the weaning day, the young mice of group C did not receive thiocyanate from water and showed an increase in daily food consumption and ingested iodide; these helped in thyroid iodine retention. Consequently, a reversible thiocyanate effect was noted, with a partial coming back of plasma thyroid hormone levels.

In contrast to iodine deficiency, where there is a preferential FT_3 rather than FT_4 production, we have noted that the FT_3 plasma level is significantly lower, whereas FT_4 is slightly reduced compared to those of group A. We can explain this result by the fact that thiocyanate has an inhibitory effect on T_4 5'-monodeiodination (DI), as demonstrated by Langer et al. [54].

On the other hand, inhibition of DI could be explained by restriction of calorific supply to mice during the critical period of weaning. In fact, we have obtained a decrease in daily food and water consumption and in body weights of 25-day-old mice. Moreover, it is well known that starvation and protein-calorie malnutrition inhibited specifically DI, inducing the reduction of plasma FT_3 levels [55,56]. It seems that the content of carbohydrate in diet as well as calorific supply maintained DI activity at the normal level.

The increase in thyroid hormone production was responsible for the decrease in TSH levels and in thyroid weights, as it was found in this investigation.

In group C, we found an important decrease in plasma TSH level comparatively to the treated group B and the control group A. This result would be explained by reversible pituitary gland hypertrophy associated with cells' transdifferentiation impairment [57].

Restoring iodide, by removing SCN^- from drinking water, ten days before sacrifice, reduced the hypertrophy of follicle epithelial cells and increased the colloidal volume. Some follicles became also larger than those of group B. In human pathologies, goitre size has been found to be reduced either by application of an iodide treatment [58], which induced a decrease in the size of cells and follicles [59], or by a reduction of plasma TSH levels, as demonstrated by our results. In fact, Van Sande et al. [60] noted that iodide uptake by the thyroid gland is competitively inhibited by SCN^- , especially high-goitrogenic substance consumption, and is alleviated with normal iodine supply [50]. This reversibility explains the return of the thyroid gland to its normal aspect.

In group C, values of consumed food and water as well as daily iodide intake were improved when compared to group B, and showed no significant difference with those of group A. This improvement would be explained by the development of their neurobehavioural activities. Indeed, after withdrawing SCN^- , the thyroid function was restored and plasma thyroid hormone levels increased, which enhanced cell multiplication in the central nervous system. Indeed, Rabie et al. [61] have demonstrated that T_4 treatment of two-week-old hypothyroid rats induced a transient wave of cell multiplication in the external granular layer of the cerebel-

lum. Therefore, according to these authors, the number of mitoses per Purkinje cell was increased by 45% after treatment with T₄ for only two days. It seems that a significant number of differentiated cells in cerebellum was increased after T₄ treatment of hypothyroid rats. Moreover, we had obtained, in a previous investigation [62], dealing with the effects of thiocyanate on the nervous system's maturation, an improvement of the biochemical parameters and of the histological aspects of cerebellum after 10 days of thiocyanate withdrawal in 25-day-old mice. Therefore, we can postulate that, during the first 15 days of life, transient hypothyroidism can occur without any effect for the neurobehavioural development of mice.

In conclusion, our study showed that, in the case of mice treated with thiocyanate, hypothyroidism effects added to the weaning event affected greatly the thyroid function and behaviour. The 25-day-old mice treated for 31 days (group B) have lower plasma thyroid hormone levels and grow less than those treated for 21 days (group C). These perturbations could be largely reversed by withdrawing thiocyanate treatment for a period of ten days.

Acknowledgements

The authors are indebted to Mrs Nabiha Mezghanni for her skillful technical assistance in radioimmunoassays. This work was supported by the DGRST grant (Appui à la recherche universitaire de base ARUB 99/UR/08-73), Tunisia.

References

- [1] H.M. Van Santen, J.E. Van Dijk, H. Rodermond, F. Vansenne, N. Meertens, J. Haveman, E. Endert, J.J. De Vijlder, T. Vulsma, The effect of cervical X-radiation on activity index of thyrocytes and plasma TSH: A pre-clinical model for radiation-induced thyroid damage, *J. Endocrinol. Invest.* 28 (2005) 261–269.
- [2] H. Bouaziz, E. Ammar, H. Ghorbel, S. Ketata, K. Jammoussi, F. Ayadi, F. Guermazi, N. Zeghal, Effects of fluoride ingested by lactating mice on the thyroid function and bone maturation of their suckling pups, *Fluoride* 37 (2004) 133–142.
- [3] H. Bouaziz, L. Soussia, F. Guermazi, N. Zeghal, Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups, *Fluoride* 38 (3) (2005) 185–192.
- [4] A. Mahjoubi-Samet, H. Fetoui, L. Soussia, F. Guermazi, N. Zeghal, Dimethoate effects on thyroid function in suckling rats, *Ann. Endocrinol.* 66 (2005) 96–104.
- [5] H. Fetoui, H. Bouaziz, A. Mahjoubi-Samet, L. Soussia, F. Guermazi, N. Zeghal, Food restriction induced thyroid changes and their reversal after refeeding in female rats and their pups, *Acta Biol. Hung.* 57 (2006) 391–402.
- [6] F. Ben Hamida, L. Soussia, F. Guermazi, N. Zeghal, Effets du thiocyanate sur la fonction thyroïdienne et la maturation du système nerveux central de la jeune souris, *Ann. Endocrinol.* 64 (2003) 268–276.
- [7] L. Soussia, F. Ben Hamida, F. Guermazi, N. Zeghal, Induction et réversibilité d'action du thiocyanate sur la fonction thyroïdienne chez le rat en période d'allaitement, *Ann. Endocrinol.* 65 (2004) 451–458.
- [8] A. Elnour, S. Lieden, P. Bourdoux, M. Eltom, S.A. Khalid, L. Hambraeus, Traditional fermentation increases goitrogenic activity in pearl millet, *Ann. Nutr. Metab.* 42 (1998) 341–349.
- [9] P. Sentisteban, M.J. Obregon, A. Rodriguez-Pena, L. Lamas, F.E. Del-Rey, G.M. De Escobar, Are iodine deficient rats euthyroid? *Endocrinology* 110 (1982) 1780–1789.
- [10] D. Glinoe, F. Delange, The potential repercussion of maternal fetal and neonatal hypothyroxinemia on the progeny, *Thyroid* 10 (2000) 871–887.
- [11] O.L. Ekpechi, Iodine deficiency disorders in Africa, in: B.S. Hetzel, J.T. Dunn, J.B. Stanbury (Eds.), *The Prevention and Control of Iodine Deficiency Disorders*, Elsevier, Amsterdam, 1987, pp. 219–236.
- [12] A.M. Ermans, C. Thilly, H.L. Vis, F. Delange, Permissive nature of iodine deficiency in the development of endemic goiter, in: J.B. Stanbury (Ed.), *Endemic Goiter*, P.A.H.O., Washington D.C., 1969, pp. 101–117.
- [13] F. Delange, The disorders induced by iodine deficiency, *Thyroid* 4 (1994) 107–128.
- [14] F. Delange, Screening for congenital hypothyroidism used as an indicator of the degree of iodine deficiency and of its control, *Thyroid* 8 (1989) 1185–1192.
- [15] A.M. Ermans, N.M. Mbulamoko, F. Delange, A. Ahluwalia, in: *Role of Cassava in the Etiology of Endemic Goiter and Cretinism*, International Development Research Centre, Ottawa, Canada, 1980, p. 136.
- [16] B. Contempré, G. Morreale de Escobar, J.-F. Denef, J.-E. Dumont, M.-C. Many, Thiocyanate induces cell necrosis and fibrosis in selenium and iodine-deficient rat thyroids: apotential experimental model for myxedematous endemic cretinism in central Africa, *Endocrinology* 145 (2004) 994–1002.
- [17] J. Cliff, D. Nicala, F. Saute, R. Givargy, G. Azambuja, A. Toela, L. Chavane, J. Howarth, Konzo associated with war in Mozambique, *Trop. Med. Int. Health* 2 (1997) 1068–1074.
- [18] C. Abuye, U. Kelbessa, S. Volde-Gebriel, Health effects of cassava consumption in south Ethiopia, *East. Afr. Med. J.* 75 (1998) 166–170.
- [19] D. Kappor, T.H. Jones, Smoking and hormones in health and endocrine disorders, *Eur. J. Endocrinol.* 152 (2005) 491–499.
- [20] A. Gasparoni, M. Autelli, M.F. Ravagni-Probizer, A. Bartoli, M. Regazzi-Bonora, G. Chirico, G. Rondini, Effect of passive smoking on thyroid function in infants, *Eur. J. Endocrinol.* 138 (1998) 379–382.
- [21] http://www.wpro.who.int/media_centre/fact_sheets/fs_20020528.htm.
- [22] J.G. Dorea, Maternal thiocyanate and thyroid status during breast-feeding, *J. Am. Coll. Nutr.* 23 (2) (2004) 97–101.
- [23] A.B. Sousa, B. Soto-Blanco, J.L. Guerra, E.T. Kimura, S.L. Gorniak, Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology* 174 (2002) 87–95.
- [24] A. Legras, D. Skrobala, Y. Furet, P. Kintz, E. Forveille, P.F. Dequin, D. Perrotin, Herbicide: Fatal ammonium thiocyanate and aminotriazole poisoning, *J. Toxicol. Clin. Toxicol.* 34 (4) (1996) 441–446.
- [25] P. Blanc, M. Hogan, K. Mallin, D. Hryhorczuk, S. Hessel, B. Bernard, Cyanide intoxication among silver-reclaiming workers, *JAMA* 253 (1985) 367–371.

- [26] H. Chandra, B.N. Gupta, S.H. Bhargave, S.H. Clerk, P.N. Mahendra, Chronic cyanide exposure—a biochemical and industrial hygiene study, *J. Anal. Toxicol.* 4 (1980) 161–165.
- [27] E.B. Sandell, I.M. Kolthoff, Microdetermination of iodine by a catalytic method, *Microchim. Acta* 1 (1937) 9–25.
- [28] K.L. Fishbeck, K.M. Rasmussen, Effect of repeated cycles on nutritional status, lactational performance and litter growth in ad-libitum fed and chronically food-restricted rats, *J. Nutr.* 117 (1987) 1967–1975.
- [29] M. Gabe, *Techniques Histologiques*, Masson, Paris, 1968, pp. 838–841.
- [30] T. Kikusui, Y. Isaka, Y. Mori, Early weaning deprives mouse pups of maternal care and decreases their maternal behaviour in adulthood, *Behav. Brain Res.* 162 (2005) 200–206.
- [31] S.J. Henning, Postnatal concentrations of total and free corticosterone during development in the rat, *Am. J. Physiol.* 235 (1976) E451–E456.
- [32] S.J. Wysocki, W. Segal, Influence of thyroid hormones on enzyme activities of myelinating rat central-nervous tissues, *J. Biochem.* 28 (1972) 183–189.
- [33] S.J. Henning, Postnatal development: coordination of feeding, digestion, and metabolism, *Am. J. Physiol.* 241 (1981) G199–G214.
- [34] M.J.O. Wakelam, C. Aragon, C. Gimenez, M.B. Allen, D.G. Walker, Thyroid hormones and precocious induction of hepatic glucokinase in the neonatal rat, *Eur. J. Biochem.* 100 (1979) 467–475.
- [35] C. Spitzweg, W. Joba, W. Eisenmenger, A.E. Heufelder, Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complimentary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa, *J. Clin. Endocrinol. Metab.* 83 (1998) 1746–1751.
- [36] N. Carrasco, Iodide transport in the thyroid gland, *Biochem. Biophys. Acta* 1154 (1993) 65–82.
- [37] G. Dai, O. Levy, N. Carrasco, Cloning and characterization of the thyroid iodide symporter, *Nature* 379 (1996) 458–460.
- [38] C.H. Bastomsky, Thyroid iodide transport, in: S.R. Geiger (Ed.), *Handbook of Physiology*, vol. 3, American Physiological Society, Washington D.C., 1974, p. 81 (sect. 7).
- [39] H. Ohtani, I.N. Rosenberg, Prompt stimulation by TSH of thyroid oxidation of thiocyanate, *Endocrinology* 88 (1971) 566–573.
- [40] C. Ohlsson, J. Isgaard, J. Törnell, A. Nillsson, O.G.P. Isaksson, A. Lindahl, Endocrine regulation of longitudinal bone growth, *Acta Paediatr.* 82 (1993) 33–40.
- [41] H. Robson, T. Siebler, S.M. Shalet, G.R. Williams, Interaction between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth, *Pediatr. Res.* 52 (2002) 137–147.
- [42] K. Ishiguro, Y. Suzuki, T. Sato, Effect of nuclear triiodothyronine (T_3) receptors in developing rat brain, *Acta Endocrinol. Copenh.* 95 (1980) 495–499.
- [43] A. Fraichard, O. Chassande, M. Plateroti, J.-P. Roux, J. Trouillas, C. Dehay, C. Legrand, K. Gauthier, M. Kedingler, L. Malaval, B. Rousset, J. Samarut, The $T3R\alpha$ gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production, *EMBO J.* 16 (14) (1997) 4412–4420.
- [44] R.J. Goody, I. Kitchen, Influence of maternal milk on functional activation of δ -opioid receptors in postnatal rats, *J. Pharmacol. Exp. Ther.* 296 (2001) 744–748.
- [45] B.Y. Muhammad, I. Kitchen, Effect of delayed weaning on opioid receptor control of swim stress-induced antinociception in the developing rat, *Br. J. Pharmacol.* 109 (1993) 651–654.
- [46] M.C. Hickey, M. Drennan, B. Earley, The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute-phase proteins and in vitro interferon-gamma production, *J. Anim. Sci.* 81 (2003) 2847–2855.
- [47] P. Nafstad, P. Magnus, B. Stray-Pedersen, Opposing placental gradients for thiocyanate and cotinine at birth, *Early Hum. Dev.* 42 (1995) 73–79.
- [48] P.M. Kuhnert, B.R. Kuhnert, P. Erhard, W.T. Brashear, S.L. Groh-Wargo, S. Webster, The effect of smoking on placental and foetal zinc status, *Am. J. Obstet. Gynecol.* 157 (5) (1987) 1241–1246.
- [49] F. Delange, Facteurs nutritionnels et fonction thyroïdienne, in: D. Lemonnier, Y. Ingenbleek (Eds.), *Les malnutritions dans les pays du Tiers-Monde*, Colloque ISERM, vol. 136, 1986, pp. 387–402.
- [50] F. Delange, Iodine deficiency as a cause of brain damage, *Postgrad. Med. J.* 77 (2001) 217–220.
- [51] W.C. Nicholas, W.F. Russell, Primary hypothyroidism presenting as a pituitary mass, *J. Miss. State Med. Assoc.* 41 (2000) 511–514.
- [52] M. Kocova, S. Netkov, E. Sukarova-Angelovska, Pituitary pseudotumor with unusual presentation reversed shortly after the introduction of thyroxine replacement therapy, *J. Pediatr. Endocrinol. Metab.* 14 (2001) 1665–1669.
- [53] J.A. Rillema, X.Y. Ting, J. Hiang, M. Sissy, Effect of prolactin on sodium iodide symporter expression in mouse mammary gland explants, *Am. J. Physiol. Endocrinol. Metab.* 279 (2000) 769–772.
- [54] P. Langer, O. Földes, K. Gschwendtova, In vivo effect of amiodarone, thiocyanate, perchlorate and goitrin on thyroxine deiodination in rat liver, *Endocrinol. Exp.* 18 (3) (1984) 177–182.
- [55] G.I. Portnay, J.T. O’Brain, J. Bush, A.G. Vagenakis, F. Aziz, R.A. Arky, S.H. Ingbar, L.E. Braverman, The effect of starvation on the concentration and binding of thyroxine and triiodothyronine in serum and on the response to TRH, *J. Clin. Endocrinol. Metab.* 39 (1974) 191–194.
- [56] I.J. Chopra, S.R. Smith, Circulating thyroid hormones and thyrotropin in adult patients with protein-calorie malnutrition, *J. Clin. Endocrinol. Metab.* 40 (1975) 221–227.
- [57] L.A. Nolan, C.K. Thomas, A. Levy, Permissive effects of thyroid hormones on rat anterior pituitary mitotic activity, *J. Endocrinol.* 180 (2004) 35–43.
- [58] Y. Li, J. Zhang, Z. Li, Prevention of iodine deficiency in high fluoride areas in Tianjin City, China, *Fluoride* (1998) 18.
- [59] R. Gartner, Thyroxine treatment of benign goiter, *Acta Med. Austriaca* 21 (2) (1994) 44–47.
- [60] J. Van Sande, C. Massart, R. Beauwens, A. Schoutens, S. Costagliola, J.E. Dumont, J. Wolff, Anion selectivity by the sodium iodide symporter, *Endocrinology* 144 (2003) 247–252.
- [61] A. Rabie, C. Favre, M.-C. Clavel, J. Legrand, Sequential effects of thyroxine on the developing cerebellum of rats made hypothyroid by propylthiouracil, *Brain Res.* 161 (1979) 469–479.
- [62] H. Ghorbel, T. Rebai, F. Guermazi, N. Zeghal, Hypothyroidism induced apoptosis in the cerebellum of weaning young mice, *Egypt. J. Biochem. Mol. Biol.* 19 (2001) 1–14.